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Detection of Extended Spectrum Beta Lactamase (ESBL) Producing Bacteria from Meat and Meat Products in Kolkata, India

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Abstract: *This cross-sectional study, was conducted on 80 samples of raw meat and meat products collected from different municipal markets and super markets in Kolkata Municipal Corporation area. The aim was to identify the prevalence of ESBL producing bacteria, which is a real threat of antibiotic resistance in human and veterinary medicine. Out of 49 pathogenic bacteria, we found two Extended Spectrum Beta Lactamase(ESBL) producers from Escherichia.coliO157. Both of the isolates were multi-drug resistant (MDR) strains. 48.97% isolates belong to Escherichia.coli and the detection rate on applying the Chi Square test was significant ($p<0.05$). The present study shows the relevance of bacterial contamination in meat and meat products as a source of gastro-intestinal infection for consumers of food of animal origin .*

Keywords: *Meat, Meat Products, E.coli, Antibiotic Sensitivity, ESBL.*

I. Introduction

There is a massive change in lifestyle, especially in urban area and non-vegetarianism in India is developing day by day. People are spending more and more to purchase products of livestock origin and they prefer ready to eat meat products. Meat hygiene is one of the most priorities of health and agricultural authorities of all countries. From public health point of view, it includes a comprehensive national food protection programme, implementation of Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP), Hazard Analysis and Critical Control Point (HACCP) system in meat inspection, surveillance and control of food borne diseases and consumer protection.[1] Processed meat and ready meals supply is increasing due to the demand for meals prepared simply and quickly(convenience food). People today are less prepared to spend the time required to cook a traditional meat meal, especially during the week days. An advantage of processed meats to the supply chain as a whole is therefore of higher value.[2] In India, meat products are manufactured both in government and private sectors. Codex Alimentarius Committee of WTO has specific recommendations for all meat products at international level.[3] Bureau of Indian Standards have developed specifications for meat products in Indian market as per public health norms.[4] It is often observed that meat products are highly contaminated, in which presence of pathogenic bacteria are also common. Itis difficult to establish whether meat products are manufactured as per the norms laid down for strict compliance with GMP and GHP. HACCP is yet to be implemented in the industry, so a threat of food borne infections is always present in our meat industry.[5]

The present study was undertaken with the objective of isolation and identification of different aerobic and anaerobic bacteria present in meat and meat products from samples collected from different municipal/super markets in Kolkata city, and their antimicrobial sensitivity pattern was enumerated. This is a great matter of concern from public health point of view and medical/veterinary microbiology.

II. Materials And Methods

2.1. Sample Collection: 80 samples were collected from different markets in Kolkata, out of which raw chicken-17, raw mutton-12, raw beef-10, frozen mutton-4, minced mutton-2, processed chicken-5, chicken salami-2, chicken momo-8, minced pork-4, pork sausage-8, pork salami-8. Samples were collected in sterile polyethylene sampling bags aseptically during a six month period (September,2011 to February,2012.). All the samples were carried to the laboratory of Department of Microbiology All India Institute of Hygiene and Public Health without any delay, where they were further processed.

2.2. Preparation of Samples: One gram of all the meat samples were minced aseptically and mixed in phosphate buffer saline (pH 7.3) to get an emulsion. The emulsion was put in culture media i,e, Nutrient Agar, Blood Agar, MacConkey Agar(MCA), Deoxycholate Citrate Agar (DCA), Thiosu lphate Citrate Bile Sucrose

Agar (TCBS), Xylose Lysine Deoxycholate Agar (XLD) media, Sorbitol MacConkey Agar and Robertson's Cooked Meat Broth and cultured in both aerobic and anaerobic conditions at 37^0 C overnight.

2.3. Isolation of Bacteria: The bacteria isolated were identified on the basis of gram staining, motility tests and standard biochemical tests.[6] E.coli O157, was identified by culturing in Sorbitol MacConkey agar and incubating plates at 37^0C for 24-48 hrs. The colony was finally identified as E.coli O157 by using Difco E.coli O157 antisera by the method of Tube Agglutination Test.[7] The kit was supplied by Becton Dickinson Company (USA). All the media used were supplied by Hi Media Laboratories, Mumbai, India.

2.4. Antibiotic Sensitivity Test: Antibiotic sensitivity test was conducted for E. coli isolates using disk diffusion techniques as described by Bauer-Kirby method by using standard disk.[8] Isolates were tested against commonly used antibiotics viz, Ampicillin (10µg), Amoxycillin(10µg), Amoxy-clav (30µg), Ciprofloxacin (30µg), Cephotaxim (30µg), Ceftazidime (30µg), Amikacin (10 µg), Cefoxitin (30µg), Cotrimoxazole (25µg) and Imipenem(10 µg). The antibiotic disks were obtained from Hi-Media Laboratories, Mumbai, India. After incubation at 37^0C overnight, diameter of the zone of inhibition were measured and results were interpreted according to CLSI guidelines [9] and recorded as susceptible(S), intermediately susceptible(I) or resistant (R) to the antibiotics.

2.5. Combined Disk Diffusion Test for ESBL production: The Combined disc diffusion test (CLSI Recommended) was followed. Test was carried out using cefotaxime(30 µg), ceftazidime(30 µg) and ceftazidime/clavulanic acid(30/10 µg) discs. The discs were obtained commercially from Hi-Media Laboratories, Mumbai, India. A positive control Klebsiella pneumonia ATCC 700603 and negative control Escherichia coli ATCC 25922 was put up along with the test. Positive results were taken when there was a =/> 5mm increase in ceftazidime/clavulanic acid zone diameter.[10]

2.6. Statistical Analysis: All collected data were later on statistically analyzed and presented. Test of proportion and Chi Square test ($\chi 2$) was used for data analysis. Probability ($p<0.05$) was considered to be significant. Statistical analysis was carried out using SPSS 14(SPSS Inc., Chicago, IL, USA).

III. Results

A total of 80 samples of raw meat and ready to eat meat products were tested in the study. Escherichia coli was detected in 26.25% of samples and Escherichia coliO157 was determined in 3.75% of samples.(Table 1) On statistical analysis detection rate of Escherichia coli was found to be significant than the other bacteria detected. Escherichia coliO157though was determined in the samples on application of χ^2test the detection rate was not significant. The number of samples showing an absence of pathogenic organisms were also significant.

42 isolates of gram negative bacilli were subjected to antibiotic susceptibility testing. The isolates were found to be most susceptible to Amoxyclav, Ciprofloxacin. Susceptibility pattern of Imipenem was 100%. Ampicillin , Amoxycillin and Cotrimoxazole showed high degree of resistance. Extended Spectrum Beta Lactamase(ESBL) was detected in 2 isolates and both of them were E.coli O157.Detection rate of ESBL was 4.65% and 66.67% of E.coli O157 produced ESBL.(Table 2). The ESBL producing bacteria were totally resistant to ampicillin, amoxicillin, cotrimoxazole, cephotaxime and amikacin. They were 100% sensitive to ciprofloxacin and imipenem.

Figure1: Colony of E.coli O157 in Sorbitol MacConkey agar

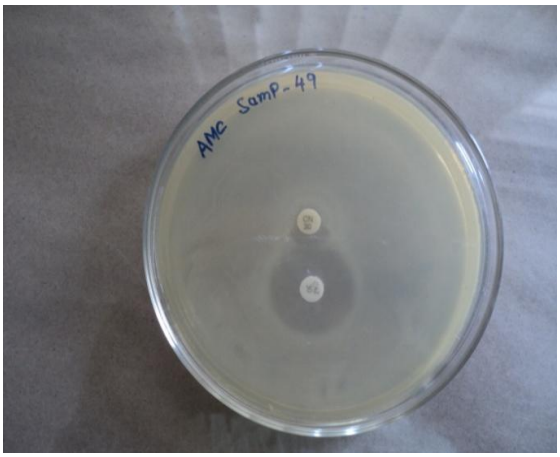

Figure2: Combined Disc Diffusion Test showing production of Extended Spectrum Beta Lactamase (ESBL).

Type of organism isolated	Number	Percentage	P value
Escherichia coli	21	26.25%	p<0.05
Escherichia coli O157	3	3.75%	p>0.05
Klebsiella spp.	8	10%	p>0.05
Staphylococcus aureus	5	6.25%	p>0.05
Pseudomonas spp	3	3.75%	p>0.05
Aeromonas hydrophila	3	3.75%	p>0.05
Enterobacter spp.	2	2.5%	p>0.05
Clostridium spp.	1	1.25%	p>0.05
Proteus vulgaris	1	1.25%	p>0.05
Proteus mirabilis	2	2.5%	p>0.05
No pathogen grown	31	38.75%	p<0.05

Table 1- Bacteria isolated from samples

Antimicrobial agents	E.coli n=21	E.coli O157 n=3	Klebsiella n=8	Pseudomonas n=3	Aeromonas n=3	Enterobactern=2	P.vulgaris n=1	P.mirabilis n=2
Ampicillin	0(0%)	0(0%)	4(50%)	2(66.6%)	0(0%)	0(0%)	0(0%)	1(50%)
Amoxycillin	0(0%)	0(0%)	5(62.5%)	2(66.6%)	0(0%)	0(0%)	1(100%)	1(50%)
Amoxy-clav	21(100%)	3(100%)	8(100%)	3(100%)	3(100%)	2(100%)	1(100%)	2(100%)
Ciprofloxacin	21(100%)	3(100%)	7(87.5%)	3(100%)	3(100%)	2(100%)	1(100%)	2(100%)
Cephotaxime	18(85.7%)	2(66.6%)	8(100%)	3(100%)	3(100%)	2(100%)	1(100%)	2(100%)
Ceftazidime	19(90.4%)	2(66.6%)	8(100%)	3(100%)	3(100%)	2(100%)	1(100%)	2(100%)
Amikacin	18(85.7%)	3(100%)	7(87.5%)	1(33.3%)	3(100%)	2(100%)	1(100%)	2(100%)
Cefoxitin	20(95.2%)	1(33.3%)	8(100%)	3(100%)	3(100%)	1(50%)	1(100%)	2(100%)
Cotrimoxazole	3(14.2%)	0(0%)	0(0%)	0(0%)	1(33.3%)	0(0%)	0(0%)	1(50%)
Imipenem	21(100%)	3(100%)	8(1000%)	3(100%)	3(100%)	2(100%)	1(100%)	2(100%)

Table2- Antibiotic susceptibility pattern of the different isolates

Figure indicates total number of isolates, number of isolates sensitive to drug and their percentages respectively.

IV. Discussion

In a country like India, where supply of protein to 120 crore population is a challenge in front of whole nation, the importance of meat and meat products cannot be overlooked. Especially, preservation of meat and its products under cold chain is absolutely essential both in retail and supermarket facility. Since meat and its products are excellent growth media for bacteria, the level and the type of contamination in meat and meat products were studied.

Out of 49 pathogenic bacteria isolated, 48.97% isolates belong to Escherichia.coli. The detection rate on applying the Chi Square test was significant ($p<0.05$). The intensity of presence of Escherichia.coli can easily be understood in meat samples from this. Out of 24 samples, 3 samples were identified as E.coli O157, by using O157 antisera in Tube agglutination test, kit supplied by Difco (USA)(Fig.1). This is important from public health point of view as E.coli O157 causes severe human disease specially acute gastroenteritis and heamorrhagic ureamic syndrome. [11] The study has a similarity with

The study conducted by Hazarika et al [12].Dutta et al determined presence of E.coli in raw fish caught from different water bodies in rural Bengal [13]. Bernard Ebang et al [14] observed that E.coli O157 strains isolated from 180 samples of meat and meat products, are highly resistant against different antibiotics, which

has a similarity with our study. The other significant finding was the absence of pathogenic bacteria in meat samples and this shows that meat hygiene was not so much compromised.

The antibiotic sensitivity test of E.coli O157 strain (two strains are ESBL producer out of 3) showed 100% sensitivity against Ceftizidime, Ciprofloxacin and Imipenem. They are completely resistant against Ampicillin, Amoxyclav, Cotrimoxazole, which is a matter of great concern from public health point of view. Extended Spectrum Beta Lactamase(ESBL) are enzymes produced by Gram negative bacteria that are capable of inactivating Penicillin, Cephalosporin's and Aztreonam. In this study we tried to establish a route of transmission of ESBL producing bacteria through food chain of human being. Two strains were isolated, producing ESBL(Fig2) and both of them were from Escherichia coli. Nagy B et al [15] observed that most of the VTEC isolates showed multi-drug resistance, which is very similar with the present study, where E.coli O157 isolates show high resistance against most of the antibiotics and were 100% sensitive to Ciprofloxacin, Amoxyclav, Amikacin and Imipenem.

V. Conclusion

The study, determines that there is a possibility that Escherichia coliO157 and ESBL producing bacteria can penetrate into the food chain of human being through livestock food products. This is a possible threat to the food industry and human health. Among the most common antibiotics, Ciprofloxacin is 100% sensitive in both the cases, which is a welcome finding from the clinical point of view. Antibiotic resistance is a burning issue nowadays and normal human flora may develop resistance by this constant exposure of resistant bacteria in the environment. These bacteria are a result of rampant antibiotic use in the food industry. Moreover all sectors of food industry may not conform to the HACCP norms. Therefore it is imperative to keep a vigil constantly by Governmental agencies and more studies are required to highlight this problem.

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